MYCOTAXON

ISSN (print) 0093-4666 (online) 2154-8889 © 2016. Mycotaxon, Ltd.

July–September 2016—Volume 131, pp. 613–619

http://dx.doi.org/10.5248/131.613

Long-hidden in Thaxter's treasure trove: *Laboulbenia camerunensis* sp. nov. parasitic on African *Curculionidae*

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Abstract—A new species, *Laboulbenia camerunensis*, parasitic on *Curculio* sp. from Cameroon, is described from a historical slide prepared by Roland Thaxter. It is the seventh species to be described from the family *Curculionidae* worldwide and the first from the African continent. The species is recognized by the characteristic outer appendage. The latter consists of two superimposed hyaline cells, separated by a black constricted septum, the suprabasal cell giving rise to two branches, the inner of which is simple and hyaline, and the outer tinged with brown. A second blackish constricted septum is found at the base of this outermost branch. Description, illustrations, and comparison to other species are given.

Key words—*Laboulbeniales*, *Laboulbeniomycetes*, insect-associated fungi, taxonomy, weevils

Introduction

The order *Laboulbeniales* (*Fungi*, *Ascomycota*, *Laboulbeniomycetes*) consists of microscopic ectoparasites that develop on the exoskeleton of certain invertebrates. The hosts are primarily beetles but also include millipedes, mites, and a variety of insects (flies, ants, cockroaches, and others). Unlike other multicellular fungi, *Laboulbeniales* exhibit determinate growth, meaning that the fungal body (thallus) develops from a two-celled ascospore through a restricted and regulated number of mitotic divisions to produce an individual with a set number of distinctively arranged cells (Tavares 1985, Santamaría 1998).

Although the earliest observations on these parasites occurred in the 1840s (Rouget 1850), it was not until the research of Roland Thaxter [1858–1932] that the *Laboulbeniales* were studied in depth. In 1890 Thaxter published the first in a series of twenty-one non-illustrated papers in which hundreds of new species were described. His five-volume illustrated monograph, published between 1896 and 1931, forms the basis for all later studies of the group. Since Thaxter died one year after the publication of the fifth volume, a planned sixth volume was never prepared. This was to have been a synthesis of the large genus *Laboulbenia* and "a general review, classification, and host-index" (Thaxter 1931). Although Thaxter left no manuscript for this final volume (Benjamin 1971), the Farlow Reference Library of Cryptogamic Botany archived his sketches and notes on undescribed species, many of which were recently re-examined along with his slide preparations.

Our recent interest in Thaxter's permanent slide collection has resulted in the description of several new species of *Laboulbeniales* on beetle hosts in the families *Carabidae* (*Laboulbenia poplitea* Haelew.), *Chrysomelidae* (*Laboulbenia bilobata* Haelew. & W. Rossi, *L. longipilis* Haelew. & W. Rossi, *L. pfisteri* Haelew. & W. Rossi), *Erotylidae* (*Laboulbenia erotylaridarum* Haelew.), and *Hydrophilidae* (*Zodiomyces rhizophorus* W. Rossi et al.) (Haelewaters & Yaakop 2014, Haelewaters & Rossi 2015, Rossi et al. 2016b). Further examination of the slides revealed yet another undescribed species of *Laboulbenia*, on *Curculio* sp., a member of the family *Curculionidae* (Wang 2014). Although reported by his contemporary Spegazzini (1917) from Argentina and more recently by Sugiyama & Majewski (1987) and Rossi et al. (2015), this is the only material studied by Thaxter that occurs on a member of the *Curculionidae*.

Currently about 2100 species of *Laboulbeniales* have been described. Species descriptions in *Laboulbeniales* are based exclusively on morphological characters, except for two species (*Hesperomyces coleomegillae* W. Rossi & A. Weir, *H. palustris* W. Rossi & A. Weir) whose morphological descriptions are supported by molecular characters (Goldmann et al. 2013). Generating sequences of *Laboulbeniales* has not been easy, although recent endeavors offer hope for advancing molecular phylogenetic research in the group when adequate fresh material is available (Haelewaters et al. 2015).

Although many authors agree that adequate species descriptions require the study of multiple juvenile and mature thalli, quite a few descriptions have been based on only one, two, or very few thalli. In these cases, morphology should be unusual and distinctive enough to make a strong case for describing the species. Examples where limited material has been used for description are *Cantharomyces magellanicus* Thaxt. (3 thalli studied, of which only one was mature, but damaged), *Dimeromyces osellae* W. Rossi (1 female and

1 male thallus), *Laboulbenia arietina* Thaxt. (4 thalli), *L. longipilis* (2 thalli), *Monoicomyces denticulatus* Thaxt. (3 thalli, of which two were mature), and *Peyritschiella xyricola* Thaxt. (1 thallus) (Haelewaters & Rossi 2015; Rossi 2010; Thaxter 1914, 1915, 1931). A more extreme case is *Peyritschiella lampropygi* Thaxt., which was described on the basis of material that was not fully developed (Thaxter 1931). For the description of the genus *Columnomyces* R.K. Benj., only a single mature (and broken) thallus was available among the 19 immature/broken thalli (Benjamin 1955).

One difficulty with our new species is that we did not have multiple adult specimens, but the available material does include one mature thallus in addition to several immature thalli. Nonetheless, because this species is easily distinguished from other *Laboulbenia* species based on the characters of the outer appendage, we describe it here as a new species. Such records help to direct collecting and ultimately inform diversity studies.

Materials & methods

Material was discovered amongst unidentified mounts studied by Roland Thaxter in the permanent slide collection, deposited at the Farlow Herbarium, Harvard University (FH).

Observations and measurements were made using an Olympus BX40 light microscope with Olympus XC50 digital camera and MicroSuite Special Edition software 3.1 (Soft Imaging Solutions GmbH). Line drawings were made using a drawing tube. Illustrations were optimized (using LEVELS and BRIGHTNESS/CONTRAST tools), cropped, and edited in Adobe Photoshop CS Version 8.0 (San Jose, California).

Taxonomy

Laboulbenia camerunensis T.W. Wang & Haelew., **sp. nov.** PLATE 1

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Differs from all other *Laboulbenia* species on *Curculionidae* by its striking outer appendage.

TYPE: Cameroon, on the abdomen of *Curculio* sp. (family *Curculionidae*, subfamily *Curculioninae*), Jun. 1912, no collector, Thaxter 2360 (**Holotype**, FH 00313482 [slide, in poor condition, partly dried out; 1 mature thallus and 10 thalli in various stages of development]).

ETYMOLOGY: The epithet *camerunensis* is derived from the country where the holotype was collected.

THALLUS hyaline, with the exception of the dark brown outermost branch of the outer appendage; 160 μm long from foot to perithecial tip. CELLS I and II of similar length, up to 3× as long as broad, with subparallel margins; forming an elongated pedestal. CELL III slightly longer than broad, distally widening. CELL IV about as long as cell III or somewhat shorter. CELL V wedge-shaped, almost

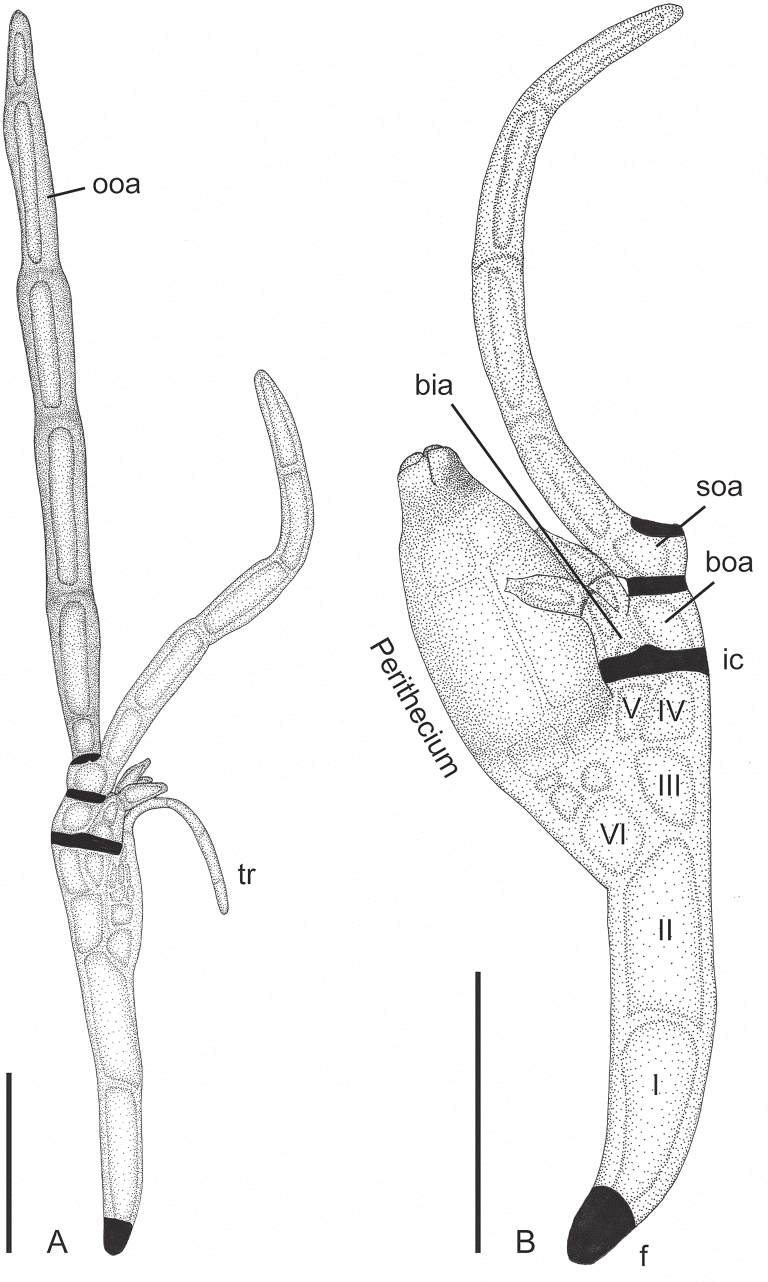
as long as cell IV; septum IV–V is oblique and reaches cell III. Insertion cell black, flattened, not marking an evident constriction on the posterior margin of the thallus. Outer appendage consisting of two superimposed almost hyaline cells separated by a black constricted septum; two simple branches arising from the suprabasal cell, composed of cells much longer than broad; the septum separating the suprabasal cell and the lower cell of the outermost branch, black, often oblique, and the area above tinged with dark brown. Inner appendage with very small basal cell, giving rise to 2–3 small cells, longer than broad, each carrying one flask-shaped antheridium, separated by a thin, dark-brown septum. Cell VI quadrangular with subequal width and length. Perithecium fusiform, largely free, 65 × 30 μm; strongly tapered at the tip, ending in four hyaline and rounded lips, two of which carry small papillae; preostiolar spots reduced to only the posterior one, which consists of a shaded area occupying the posterior lip.

Discussion

We think it is safe to assume that the host genus is correctly identified as *Curculio* Linnaeus 1758, according to current taxonomical concepts. The superfamily *Curculionoidea* (*Coleoptera*) comprises over 60,000 species of weevils, most of which represent the *Curculionidae* (true weevils). The phylogenetic classification of this family is complex, leading to recent debate (Jordal et al. 2014). However, the taxonomy of the *Curculionini* tribe in Africa has remained almost unchanged since Thaxter's work (Löbl & Smetana 2013, Caldara et al. 2014).

Six *Laboulbenia* species were previously described from *Curculionidae: L. curculionidicola* K. Sugiy. & T. Majewski from Peru; *L. dichroma* W. Rossi et al. on *Geraeus* sp. from Ecuador; *L. elephantina* W. Rossi et al. on *Udeus* sp. from Ecuador; *L. hyemalis* Speg. on *Anthonomus* sp. from Argentina; *L. inopinata* W. Rossi et al. on *Lechriops* spp. and *Macrocopturus* spp. from Ecuador; and *L. microcarpa* W. Rossi et al. on *Sicoderus* sp. and *Lancearius esau* from Ecuador (Spegazzini 1917, Sugiyama & Majewski 1987, Rossi et al. 2015). *Laboulbenia camerunensis* can be easily distinguished from these species by its characteristic

Plate 1. *Laboulbenia camerunensis* (holotype, FH 00313482). A. Juvenile thallus, with trichogyne (*tr*), an external thin appendage-like outgrowth of the young perithecium through which fertilization occurs. B. Mature thallus, with the outermost branch of the outer appendage broken off above the suprabasal cell (*soa*). Scale bars = 50 μm. Labeled are: the foot (*f*) with which the thallus attaches to the host's integument; cells I, II, III, IV, and V of the receptacle; the insertion cell (*ic*); the basal cell (*boa*), and suprabasal cell (*soa*) of the outer appendage, as well as its outermost branch (*ooa*) [only in A]; the very small basal cell of the inner appendage (*bia*), which carries 2 [in B] or 3 [in A] cells each supporting a single antheridium; and cell VI, carrying the perithecium (including basal cells).


ooa
tr
A
bia
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Perithecium
V
IV
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VI
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I
f
B

outer appendage structure, in particular the two dark septa at the base and the outermost branch tinged with dark brown above its lower cell.

Laboulbenia curculionidicola is additionally distinguished from *L. camerunensis* by cell II being twice as long as cell I, cell III usually splitting into two, cell V located in the upper inner corner of cell IV, and the outer appendage comprising a simple branch. *Laboulbenia hyemalis* is further distinguished by its outer appendage consisting of a single main axis that is blackened only at the posterior margin and its perithecial tip distinctly curved toward the anterior side. *Laboulbenia dichroma*, *L. elephantina*, and *L. microcarpa*, among other differences, have a simple, unbranched outer appendage. The final species, *L. inopinata*, differs greatly from all other species on *Curculionidae* in its undivided androstichum (= cells III + IV + V) and very simple appendage structure. The undivided androstichum is actually typical for species of *Laboulbenia* on *Chrysomelidae* (Rossi et al. 2016a).

The outer appendage of *L. camerunensis,* highly distinctive in *Laboulbenia*, superficially resembles *L. pfisteri*, which, however, differs in many other characteristics: (i) cell V does not reach cell III, (ii) the outer appendage has an additional third branch borne on the inner angle of the basal cell, and (iii) the perithecium is noticeably enlarged in its upper portion (Haelewaters & Rossi 2015).

Roland Thaxter's original sketch of this species, obtained from the archives of the Farlow Reference Library of Cryptogamic Botany, was published in Wang (2014: 3).

Acknowledgments

We wish to thank Lisa Decesare, Head of Archives and Public Services at the Botany Libraries, Harvard University, for her efforts to access Roland Thaxter's unfinished drawings in the archives of the Farlow Reference Library. We also thank Kanchi N. Ghandhi for nomenclatural advice, Meredith Blackwell and Walter P. Pfliegler for reviewing the manuscript, and Shaun R. Pennycook for additional edits and suggestions.

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